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Regulation of apple leaf aldose-6-phosphate reductase activity by inorganic phosphate and divalent cations

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Abstract. Aldose-6-phosphate reductase (A6PR), a key enzyme in sorbitol biosynthesis, has been purified to apparent homogeneity from fully developed apple (*Malus domestica* Borkh. cv. Gala) leaves. Inorganic phosphate inhibited A6PR by decreasing the maximum velocity of the enzyme and by increasing the K_m for the substrate, glucose-6-phosphate (Glc6P). Divalent cations including Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+} altered A6PR activity. Effects of Ca^{2+} and Mg^{2+} on A6PR activity were dependent upon both the metal ion concentration and the concentration of Glc6P. The activity of A6PR was increased by 0.5–5 mM Ca^{2+} or Mg^{2+} when Glc6P concentration was below 10 mM. However, these same metal ions decreased A6PR activity at greater Glc6P concentrations or in the presence of higher metal ion concentrations. A6PR displayed Michaelis–Menten kinetics either in the presence or absence of 2.5 mM $MgCl_2$, but the apparent K_m for Glc6P decreased from 11.3 mM for the control to 5.1 mM in the presence of 2.5 mM $MgCl_2$ in the assay mixture. By contrast, Zn^{2+} and Cu^{2+} dramatically inactivated A6PR activity. A6PR activity was decreased approximately 50 and 70%, respectively, when the enzyme was pre-incubated with 2 mM Zn^{2+} or Cu^{2+} for 60 min at room temperature. This inactivation was partially reversed by dialysis or by chelation with 20 mM EDTA. NADPH and $NADP^+$, which are substrates for A6PR in the oxidative and reductive directions, respectively, partially protected A6PR from inactivation by Zn^{2+}. The above results suggest that both Mg^{2+} and Ca^{2+} were mixed-type, non-essential activators of A6PR that decreased the K_m for sugar-phosphates but lowered the overall V_{max}. The physiological significance of these findings is also discussed.

Keywords: aldose-6-phosphate reductase, apple, carbohydrate metabolism, divalent ions, inorganic phosphate, *Malus domestica*, sorbitol synthesis.

Introduction

There has been considerable interest in elucidating the regulatory mechanisms of carbohydrate metabolism in plants because carbohydrates are the main determinants of agricultural crop yield (Gifford *et al*. 1984; Koßmann *et al.* 1996). For most plants, sucrose and starch are the principal end-products of photosynthesis. The biochemical and molecular regulation of sucrose and starch metabolism has been studied extensively (Preiss and Sivak 1996; Quick and Schaffer 1996). In contrast to the majority of plant species, sorbitol serves as the primary photosynthetic end-product for certain woody species of the Rosaceae (Bieleski 1982; Loescher and Everard 1996). Sorbitol is the predominant storage and transport carbohydrate in these plant species (Loescher and Everard 1996). It has been suggested that sorbitol plays a role in stress tolerance of plants. Prior studies revealed that sorbitol accumulated during water stress (Wang and Stutte 1992; Lo Bianco *et al*. 2000) and sorbitol has also been shown to ameliorate cell damage during salt stress (Shen *et al*. 1999). Sorbitol may also facilitate the uptake and transport of boron in plants (Brown *et al*. 1999).

Aldose-6-phosphate reductase (A6PR), alternatively named NADP-dependent sorbitol-6-phosphate dehydrogenase, catalyses the reversible reaction of the reduction of Glc6P to sorbitol-6-phosphate using NADPH as a co-factor. The synthesized sorbitol-6-phosphate is subsequently hydrolyzed to release free sorbitol (Zhou *et al*. 2003). Developmental and seasonal studies of apple and peach leaves have shown that increased A6PR activity was

Abbreviations used: A6PR, aldose-6-phosphate reductase; ES, enzyme–substrate; ESA, enzyme–substrate–activator; Glc6P, glucose-6-phosphate; DTT, dithiothreitol; P_i, inorganic phosphate; SPS, sucrose phosphate synthase.

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correlated with the development of photosynthetic capacity and sorbitol accumulation (Loescher *et al*. 1982; Yamaki and Ishikawa 1988; Merlo and Passera 1991). Transgenic techniques confirmed the key role of A6PR in sorbitol biosynthesis. Tobacco plants that normally do not manufacture polyols were engineered to produce sorbitol by the insertion of cDNA sequences encoding A6PR. This observation conclusively demonstrated that A6PR activity was sufficient for the formation of sorbitol (Tao *et al*. 1995; Sheveleva *et al*.1998). Similar results were also obtained with transformed yeast (Shen *et al*. 1999) and persimmon (Gao *et al*. 2001).

Compared with the abundant literature on sucrose and starch metabolism, relatively few studies have been performed on the regulation of sorbitol synthesis. Properties of purified A6PR have only been examined in a limited number of prior studies (Hirai 1981; Negm and Loescher 1981; Kanayama and Yamaki 1993). These earlier studies suggested that the affinity of A6PR for Glc6P was very low, with an apparent K_m for Glc6P of more than 10 mM, which cannot explain the abundant sorbitol synthesis in apple leaves. ATP and ADP have been demonstrated to be competitive inhibitors with K_i values of 0.l8 mM and 0.30 mM, respectively (Kanayama and Yamaki 1993), but the physiological significance of this inhibition of A6PR when the cellular energy status is high remains unclear. In order to better understand the regulatory mechanisms of sorbitol synthesis, we investigated the effects of metabolites and cations on A6PR activity in apple leaves. We report that P_i inhibited A6PR by decreasing the V_{max} and increasing the K_m for Glc6P. The divalent metal ions, Ca^{2+} and Mg^{2+} increased A6PR activity by lowering the K_m for Glc6P. Conversely, Zn^{2+} and Cu^{2+} inhibited A6PR activity.

Materials and methods

Materials

Young apple trees (*Malus domestica* Borkh. cv. Gala) were pruned to two shoots per plant in the early spring and grown at the Cornell experimental orchard under natural environments. The trees were watered twice each day and fertilized weekly with Peters® (Scotts–Sierra Horticultural Products, Marysville, OH) water-soluble 20N–20P–20K water-soluble fertiliser. Fully expanded source leaves from up to 10 plants were harvested at mid-day and enzyme extractions were performed immediately.

Assay of enzyme activity

A6PR was assayed in the direction of sorbitol-6-phosphate synthesis by following the oxidation of NADPH in the presence of Glc6P (Zhou *et al*. 2001), The reaction mixture (1 mL) contained 0.1 M Tris–HCl (pH 9.0), 0.1 mM NADPH, 50 mM Glc6P and 50 µL of the extract. Changes in absorbance at 340 nm were monitored between 0–3 min and the oxidation rate of NADPH was calculated using an absorption coefficient of 6.22 mM^{-1}.

Purification of aldose-6-phosphate reductase

All purification steps were performed at 4°C. Column chromatography was performed with an ÅKTA protein purification system from Amersham Biosciences (Piscataway, NJ). Approximately 200 g of freshly harvested apple mature leaves were homogenized at full line voltage in a Waring blender with 1 L of extraction buffer containing 0.1 M Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM DTT, 5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP), and 0.01% (v/v) Triton-X 100. The homogenate was filtered through four layers of cheesecloth and the filtrate was centrifuged at 12000 *g* for 10 min. Solid ammonium sulfate was slowly added to the supernatant to attain 40% saturation with constant stirring for 30 min. The resultant suspension was centrifuged at 12000 *g* for 10 min. The supernatant was slowly brought to 60% saturation with solid ammonium sulfate as described above. The sample was centrifuged as above and the 40–70% pellet was resuspended in 50 mL extraction buffer minus PVPP and Triton-X 100. The resultant solution was clarified by centrifugation at 12000 *g* for 5 min and the supernatant was desalted using PD10 columns (Amersham Biosciences) with 10 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$, 0.1 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 mM DTT (buffer A). The desalted preparation was then applied directly to a 2.5 × 30 cm DEAE–cellulose column pre-equilibrated with buffer A. After washing the column with two bed-volumes of buffer A, the enzyme was eluted with five bed-volumes of buffer A in a linear gradient of KCl from 0–1 M at a flow rate of 1.2 mL min^{-1}. Fractions containing A6PR activity were pooled and condensed with a dialysis bag against PEG 8000. An aliquot of the concentrated enzyme preparation was loaded to a Mono Q HR5/5 anion-exchange column (Amersham Biosciences). The column was rinsed with 4 mL buffer A and the enzyme was then eluted at 1 mL min^{-1} with 20 mL buffer A in a linear gradient of KCl from 0–1 M. Fractions with the highest A6PR activity were pooled and desalted as above and loaded directly onto a column of Blue Sepharose CL-6B (1 × 7 cm) that had been previously equilibrated with buffer A. The column was washed with 15 mL buffer A containing 0.14 M KCl, and then the enzyme was eluted with buffer A containing 0.28 M KCl. The subsequent active fractions were collected and concentrated in a dialysis bag against solid PEG 8000 and then loaded onto a Superose 6 column (Amersham Biosciences) equilibrated with 20 mM Tris–HCl (pH 8.0) containing 0.2 mM dithiothreitol (DTT). The enzyme was eluted with column buffer using a flow rate of 0.25 mL min^{-1}. Active fractions containing purified A6PR were pooled and were stored at –80ºC.

Electrophoresis

Samples were analysed by SDS–PAGE using 7 × 10 cm mini-gels prepared with a 12% (w/v) acrylamide resolving gel according to Laemmli (1970). Samples were mixed with an equal volume of buffer containing 0.3 M Tris–HCl (pH 6.8), 3.3% SDS, 0.15 M DTT, and 33% glycerol and were denatured in a boiling water bath for 2 min. Electrophoresis was performed for 100 min at 150 V. Protein bands were located by staining with a silver staining kit (ProtSil-1, Sigma Chem., St Louis, MO), according to the manufacturer's directions.

Protein assay

Protein concentration was measured according to Bradford (1976) using bovine serum albumin as the protein standard.

Estimation of the molecular mass of A6PR

Gel filtration was carried out using the purified A6PR. The enzyme was loaded on to a Superose 6 column (10 × 30 cm) (Amersham Biosciences) equilibrated with buffer A. The molecular mass was determined using the standard curve derived from standard proteins. Molecular mass of A6PR was also determined by SDS–PAGE (12%) using the standard curve derived from standard proteins.

Table 1. Purification of A6PR from mature apple leaves

Step	Protein (mg)	Specific activity (unit mg^{-1})	Purification (fold)	Yield (%)
Crude extraction	1460	0.0084	1	100
$(NH_4)_2SO_4$ (40–70%)	572	0.02	2.4	92
DEAE Cellulose	64.3	0.15	17.3	79
Mono Q	12.0	0.71	85	70
Blue Sepharose	0.84	1.65	196	57
Superose 6	0.48	7.94	945	31

Results

Purification of A6PR

The purification procedure for A6PR led to a 945-fold increase in specific activity over that in the crude extract, and the overall recovery was 31% (Table 1). The preparations appeared homogeneous and yielded a single band with a molecular mass of 33 kDa in SDS–PAGE with silver staining (Fig. 1). Native molecular mass was determined to be 68 kDa by Superose 6 gel-filtration chromatography. These results suggest that apple A6PR is a homodimer with a subunit of 33 kDa. The specific activity of the purified enzyme was 7.94 μmol min^{-1} mg^{-1} protein.

Stimulation and inhibition of A6PR activity by metal ions

As shown in Table 2, divalent ions including Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} affected A6PR activity. At a concentration of 2.5 mM, $MgCl_2$ and $CaCl_2$ increased A6PR activity by 40% and 52%, respectively, when the enzyme activity was determined at low substrate concentrations (5 mM Glc6P and 100 μM NADPH in 50 mM Tris–HCl, pH 9.0). These same metal ions reduced A6PR activity approximately 12–14% when the activity was assayed at high Glc6P concentration (50 mM). These cation effects on A6PR activity were also observed when bis-Tris–propane–HCl (pH 9.0) was substituted for Tris–HCl (pH 9.0) in the enzyme reaction (data not shown).

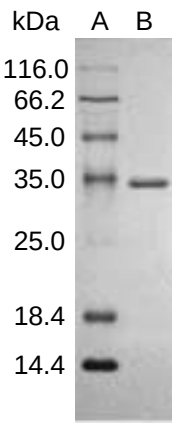

Fig. 1. SDS–PAGE of purified apple A6PR. Protein was subjected to SDS–PAGE in a 12% polyacrylamide gel and stained with silver stain plus. Lane A, standard markers (kDa); lane B, purified A6PR.

Effects of divalent metal ions on A6PR activity were concentration dependent. The stimulation of A6PR activity by Ca^{2+} was greatest at 1 mM, whereas Mg^{2+} stimulated A6PR activity in the range of 0.5–2.5 mM. Higher levels of Ca^{2+} or Mg^{2+} in the assay mixture inhibited A6PR activity (data not shown).

$ZnCl_2$ and $CuCl_2$ inhibited the A6PR activity at both lower and higher Glc6P concentrations. Including 1 mM $ZnCl_2$ or $CuCl_2$ in the assay mixture decreased the enzyme activity by 35–48% (Table 2). No significant alterations in A6PR activity were observed with 5 mM KCl added to the assay mixture. This result indicates that the inhibitory effects of metal salts on A6PR activity were caused by the divalent metal ions rather than Cl^-.

To further study the activating effects of metal ions on A6PR activity, we analysed the kinetics of A6PR preparations in the presence or absence of 2.5 mM $MgCl_2$. Our result shows that both the control and Mg^{2+}-treated A6PR displayed hyperbolic kinetics against Glc6P. The inclusion of 2.5 mM Mg^{2+} reduced the K_m of A6PR for Glc6P from 9.8 to 5.1 mM (Fig. 2). The addition of 2.5 mM $MgCl_2$ also altered A6PR activity in the direction of sorbitol-6-phosphate oxidation. The K_m for sorbitol 6-phosphate decreased from 3.0 to 1.7 mM when 2.5 mM Mg^{2+} was added to the assay mixture (data not shown).

Table 2. Effect of some cations on purified apple A6PR activity
Enzyme activity was determined with 100 mM Tris–HCl, pH 9.0, 0.1 mM NADPH and Glc6P as indicated in the table. All values are the mean of four independent measurements with a standard error of less than 5% of the mean

	Activity (nmol min^{-1} mL^{-1})	
	[Glc6P] = 5 mM	[Glc6P] = 50 mM
Control	1.13	3.14
2.5 mM $CaCl_2$	1.92	2.74
1.0 mM $CuCl_2$	0.58	1.62
2.5 mM $MgCl_2$	1.58	2.70
1.0 mM $ZnCl_2$	0.61	2.03
5.0 mM KCl	1.11	3.10

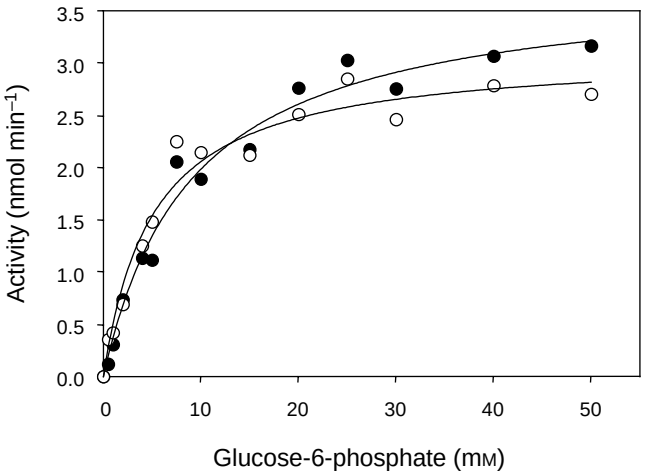

Fig. 2. The A6PR-catalysed reaction velocity *v.* the concentration of Glu6P in the absence (●) and in the presence (○) of 2.5 mM $MgCl_2$.

Inhibition of A6PR by inorganic phosphate

Inorganic phosphate (P_i) was found to be a mixed type of inhibitor for A6PR with a K_i of 0.42 mM in the current study. As the P_i concentration increased from 0 to 4 mM, the maximum velocity of A6PR catalysing the reduction of Glc6P decreased from 3.6 to 2.3 nmol min^{-1} mg^{-1} protein. The corresponding K_m for Glc6P increased from 5.1 mM to 11.8 mM when P_i levels in the assay increased from 0 to 4 mM (Fig. 3).

Inactivation of A6PR by Zn^{2+}

Pre-incubation of A6PR with $ZnCl_2$ at 25°C before assay inhibited the enzyme's activity. These cation effects were

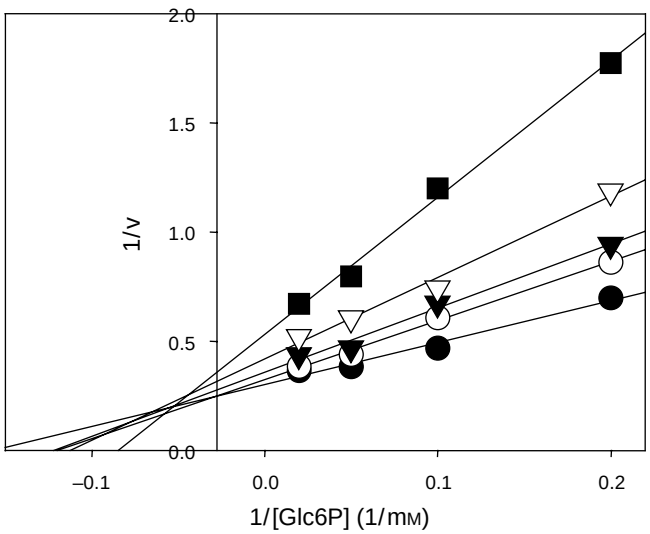

Fig. 3. The effect of P_i on apple leaf A6PR activity. The experiment was repeated three times and the same pattern was observed. Typical data are presented. The treatment includes control without P_i (●), 1 mM P_i (○), 2 mM P_i (▲), 3 mM P_i (△) and 4 mM P_i (■).

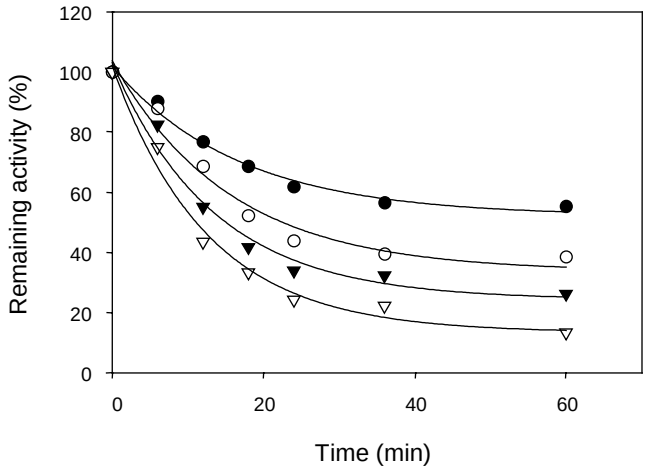

Fig. 4. Inactivation of A6PR by Zn^{2+}. The A6PR preparation was pre-incubated with $ZnCl_2$ in 20 mM Tris–HCl (pH 8.0) containing 0.2 mM DTT at the indicated concentration at 25°C. A portion was withdrawn at the indicated time and diluted 100-fold to measure the enzyme activity. The relative activity was calculated as the ratio of the remaining activity with and without Zn^{2+} pre-incubation treatments. $ZnCl_2$ concentrations used in the study are 0.5 mM (●), 1 mM (○), 2 mM (▲), 3 mM (△).

greater with increasing duration of the pre-incubation and with increasing Zn^{2+} concentrations. A6PR activity was decreased by 31, 48, 58 and 67% after 18 min of incubation with 0.5, 1, 2, and 3 mM Zn^{2+} at 25°C, respectively (Fig. 4). Similar results were obtained when A6PR was pre-incubated with Cu^{2+} (data not shown). The inactivation of A6PR by Zn^{2+} was partially reversible. After A6PR was pre-incubated with 2 mM $ZnCl_2$ for 60 min at 25°C, the enzyme lost 63% of its activity. The subsequent addition of 20 mM EDTA or dialysis against 20 mM Tris–HCl (pH 8.0) containing 0.2 mM DTT overnight at 4°C, restored most of the lost activity (Table 3).

Metabolite protection of A6PR inactivation by Zn^{2+}

Various metabolites affected the inactivation of A6PR by Zn^{2+} (Table 4). The effects of six metabolites, ATP and ADP (competitive inhibitors) (Kanayama and Yamaki 1993), Glc6P, sorbitol-6-phosphate (sor6P), NADPH, and $NADP^+$ (substrates) on enzyme inactivation by Zn^{2+} were evaluated

Table 3. Recovery of zinc-inactivated A6PR activity by dialysis and chelation

Relative activity was calculated as the percentage of the original activity, which was the activity before Zn^{2+} treatment. All values are the mean of four independent measurements with a standard error of less than 5% of the mean

Treatment	Relative activity (%)
Control	37
20 mM EDTA	63
Dialysis	78

in this study. Initially, A6PR was mixed with metabolites for 2 min, and then 2 mM $ZnCl_2$ was added to inactivate the enzyme as described above. NADPH and $NADP^+$ partially protected A6PR from inactivation by Zn^{2+}. After pre-incubation with 1 mM NADPH or $NADP^+$, A6PR activity decreased 7% and 11%, respectively, after a 10 min exposure to 2 mM $ZnCl_2$ treatment, while only 50% of the enzyme's activity remained after Zn^{2+} pre-treatment without prior incubation with NADPH or $NADP^+$. ATP and ADP also demonstrated a small protective effect against Zn^{2+}-induced inactivation. The greatest loss of A6PR activity occurred when the enzyme was pre-treated with 5 mM Glc6P before the addition of Zn^{2+}. Pre-incubation with 5 mM sorbitol-6-phosphate provided no protection against the Zn^{2+}-dependent inactivation of A6PR.

Discussion

Other authors (Hirai 1981; Negm and Loescher 1981; Kanayama and Yamaki 1993) demonstrated that apple leaves contained a specific dehydrogenase that catalysed the reversible NADPH-dependent reduction of Glc6P to sorbitol-6-phosphate. The enzyme was purified 79- to 1800-fold, produced a single band at 36 kDa on denaturing gels, and was shown to be a dimer. Results obtained with A6PR preparations in the present study were consistent with these earlier studies.

Aldose-6-phosphate reductase is the key enzyme catalysing sorbitol biosynthesis in several economically important woody Rosaceae species. Unfortunately, very little is known about the regulatory mechanisms controlling the activity of this enzyme *in vivo*. Current evidence suggests that sorbitol synthesis and sucrose synthesis occur in the cytosolic compartment of apple leaf mesophyll cells. Since both of these metabolic pathways ultimately depend upon a single pool of hexose-phosphates to supply substrates, it is intuitively obvious that metabolic controls are necessary to coordinately control the simultaneous biosynthesis of sucrose and sorbitol. The finding that A6PR activity is inhibited by P_i may provide a reasonable mechanism for the metabolic fine control of A6PR in relation to the rate of photosynthesis. As indicated in Fig. 5, P_i is stoichiometrically exchanged for triose-phosphate at the inner membrane of chloroplast; thus, an accumulation of P_i in the cytosol could signal a reduction in the supply of carbon from the chloroplast. An increase of P_i levels in the cytosol would correspond with decreased concentrations of phosphate esters such as Glc6P. Conversely, a high photosynthetic rate might be associated with decreased P_i and increased phosphate ester concentrations. Consequently small reciprocal changes in P_i and Glc6P potentially would have a large and significant effect on A6PR activity.

Sucrose and starch are the primary photosynthetic end-products in most plants. P_i has been shown to regulate several enzymes involved in sucrose and starch synthesis. Sucrose phosphate synthase (SPS), which is responsible for sucrose synthesis in plants is allosterically activated by Glc6P and inhibited by P_i (Huber and Huber 1996). Note that the inhibition of SPS by P_i also occurs at millimolar concentrations. P_i also allosterically inhibits adenosine diphosphate glucose pyrophosphorylase, the principal control point for starch synthesis in the plastid (Preiss and Sivak 1996). The coordination of carbon assimilation and the photosynthate formation in plant cells is, at least partially, achieved by P_i inhibition of sucrose and starch synthesis. Interestingly, our previous results indicate that apple leaf SPS is not very sensitive to P_i inhibition (Zhou *et al*. 2002).

Previous studies showed that the K_m of A6PR for Glc6P was greater than 10 mM (Hirai 1981; Negm and Loescher 1981; Kanayama and Yamaki 1993). Glc6P is an important metabolite in the cell and is used in several pivotal biochemical processes such as glycolysis, sucrose synthesis, and the reductive pentose phosphate pathway. The previously reported lower affinity of A6PR for Glc6P is not consistent with the fact that a large percentage of photosynthetically fixed carbon in apple source leaves is partitioned into sorbitol (Wang and Stutte 1992). Chen *et al*. (2002) demonstrated that phosphoenolpyruvate carboxykinase could operate as a co-carboxylase *in vivo* at physiological concentrations of metal ions. This enzyme was previously thought to be a decarboxylase, based on its low affinity for CO_2, which was determined at non-physiological conditions. Results of the current study demonstrated that Mg^{2+} increased A6PR activity at low Glc6P and that 2.5 mM Mg^{2+} decreased the K_m for Glc6P. It is of interest to note that the concentration of Mg^{2+} in the cytosol of mung bean roots was reported to be in the millimolar range (Yazaki *et al*. 1988). Information is not available on Mg^{2+} concentration in the cytosol of apple leaf cells. The reduced

Table 4. Effect of pre-treatment with metabolites on the inactivation of A6PR by Zn^{2+}

Control represents A6PR activity as described in Materials and methods without added metabolites or metal ions. Zn-inactivated represents A6PR activity after pre-treatment with 2 mM Zn^{2+} for 10 min at 25°C before initiating the assay. In all other treatments, A6PR was first pre-incubated with specific metabolite for 2 min at 25°C before the addition of 2 mM $ZnCl_2$ for 10 min. All values are the mean of four independent measurements with a standard error of less than 5% of the mean

Treatment	Relative activity
Control	100
Zn-inactivated	50
3 mM ADP	59
3 mM ATP	64
5 mM Glc6P	35
1 mM NADP	89
1 mM NADPH	93
5 mM Sor6P	52

K_m of A6PR in the presence of Mg^{2+} would facilitate the synthesis of sorbitol in apple leaves when Glc6P concentrations were low.

Metal ions can activate an enzyme by a variety of mechanisms (Dixon and Weber 1979). The present results suggested that Mg^{2+} and Ca^{2+} acted as mixed-type activators with respect to A6PR. This conclusion is based on the observation that these ions stimulated A6PR activity at low Glc6P concentrations but inhibited A6PR when Glc6-P levels were high. Cations involved in this process are also referred to as mixed-type inhibitors because at high substrate concentrations the V_{max} of the enzyme is decreased.

Mixed-type activators increase the affinity of an enzyme for its substrate, but decrease the rate constant for product formation (Segal 1975). At low substrate concentration, there is a higher concentration of enzyme–substrate–activator (ESA) complex than of enzyme–substrate (ES) complex. At low substrate levels greater concentrations of ESA more than compensate for the fact that ESA is not as effective as ES in forming product. However, as the substrate concentration increases the advantage of increased substrate affinity in the presence of the activator is lost and the V_{max} is decreased.

Our result showing that Zn^{2+}- and Cu^{2+}-inactivated A6PR has potential important mechanistic considerations. Thiolation by Zn^{2+} can reduce an enzyme's activity if the thiol group is involved in the catalytic reaction or if a conformational change occurs to the protein rendering the active site less accessible (Van Assche and Clijsters 1990; Lindstad and McKinley-McKee 1996). Many Zn^{2+}-inactivated enzymes in plants have been identified and inactivation typically resulted from the interaction of Zn^{2+} with a thiol group at the active centre (van Assche and Clijsters 1990). Negm and Loescher's results (1981) also indicated that A6PR contained key thiol group(s). Pre-incubation with $NADP^+$ or NADPH largely protected A6PR from Zn^{2+} inactivation. Because NADPH and $NADP^+$ are substrates for A6PR, it is logical to assume that the key thiol group(s) is present in or near the NADP/NADPH binding site(s). We feel that the binding of NADP/NADPH with A6PR prevents Zn^{2+} from reacting with the key thiol groups.

In summary, the above findings indicated that the activity of A6PR was subject to complex control mechanisms primarily involving concentrations of P_i, Glc6P and divalent cations. These results likely are indicative of the crucial role that A6PR performs in regulating

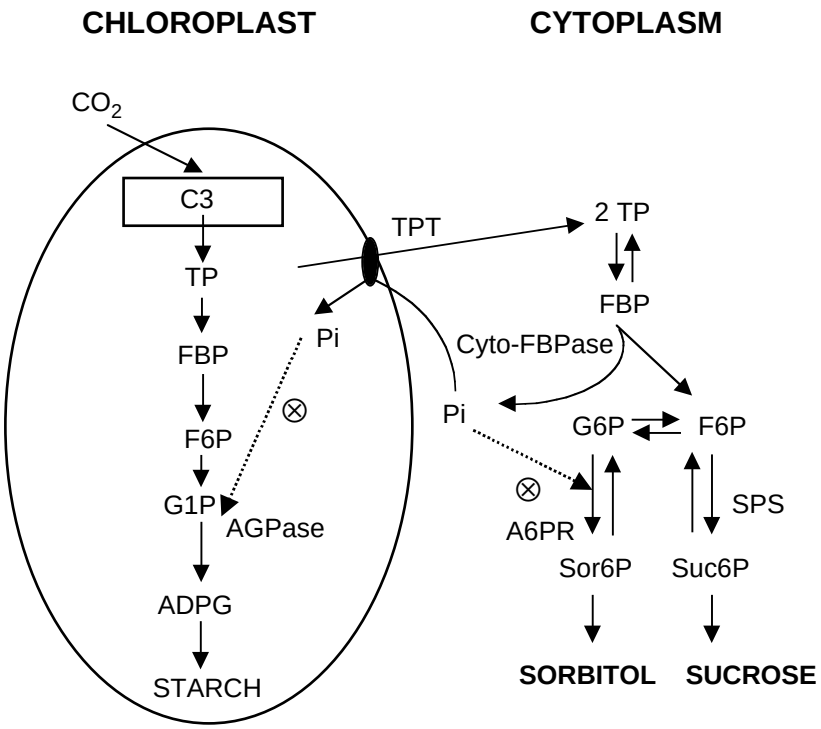

Fig. 5. Schematic representation of P_i effects on carbohydrate biosynthesis in leaves of sorbitol-synthesizing species. The dashed lines and the symbol ⊗ represent the inhibitory effect. ADPG, adenosine diphosphate glucose; A6PR, aldose-6-phosphate reductase; cyto-FBPase, cytosolic fructose-1,6-bisphosphatase; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; Pi, inorganic phosphate; SPS, sucrose-phosphate synthase; Sor6P, sorbitol-6-phosphate; Suc6P, sucrose-6-phosphate; TP, triose phosphate; TPT, triose-phosphate transporter.

photosynthetic C metabolism in apple leaves and in related sorbitol-forming species.

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